

Standardization of an immunochemistry staining to detect amyloid material in domestic species

Maria Riera Augé - June 2016



Objectives

- To review the current knowledge of the amyloidosis in domestic and wild animals.
- To investigate the current knowledge of the amyloidosis as a potentially transmissible disease.
- To standardize an immunochemistry staining to detect amyloid material.
- To evaluate the immunochemistry staining in different domestic and wild species.

Materials and methods

Selection of the species

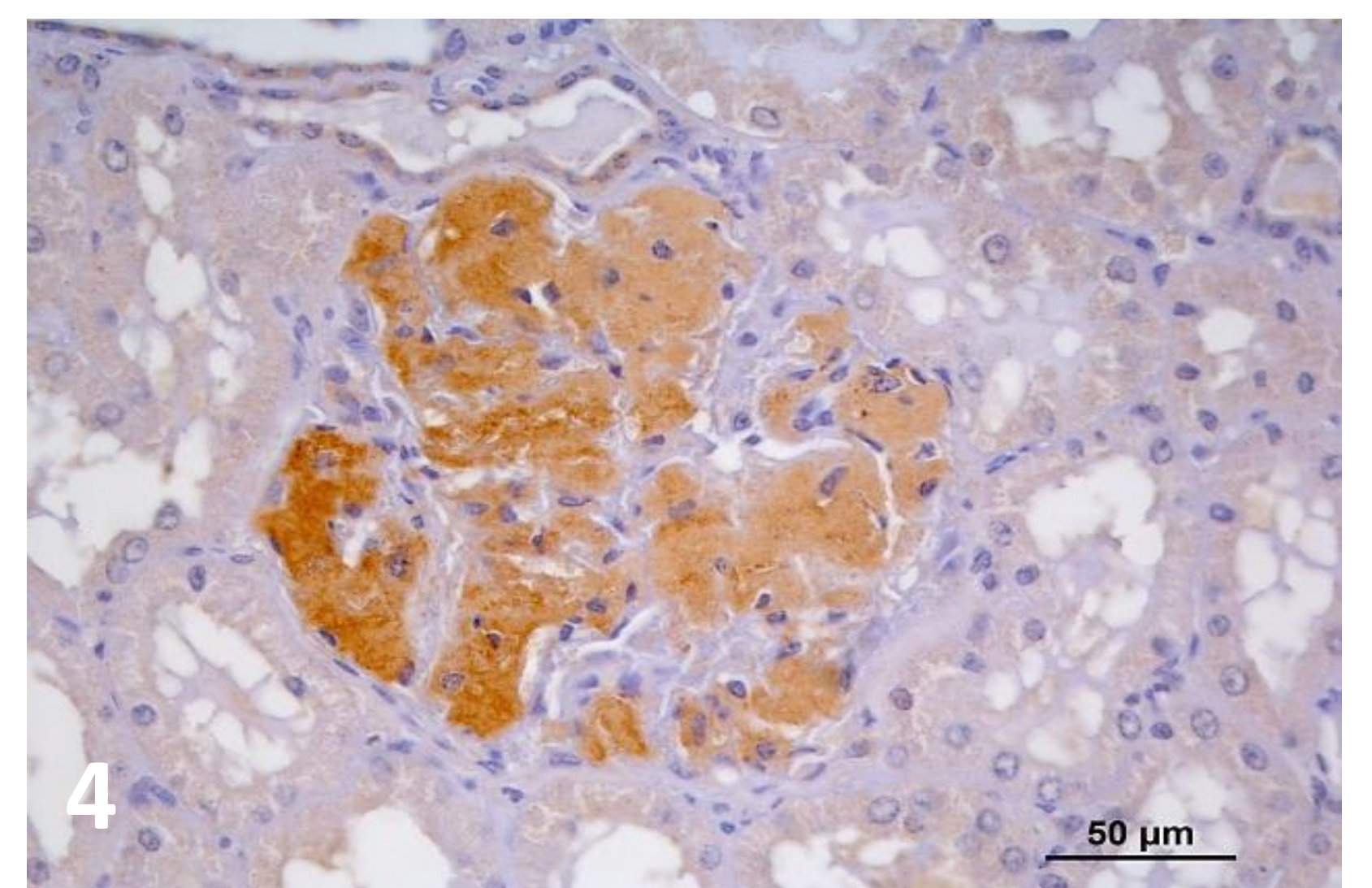
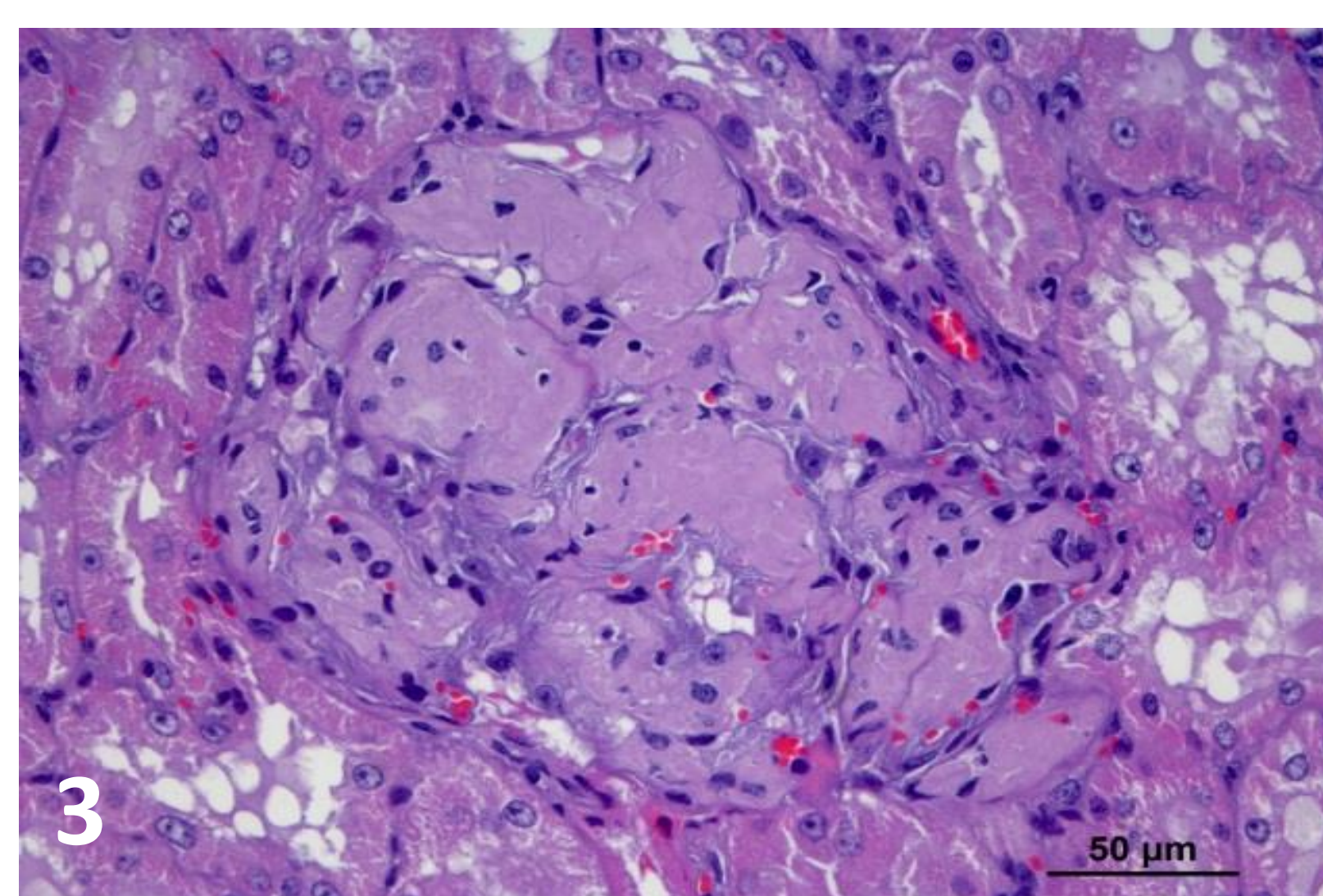
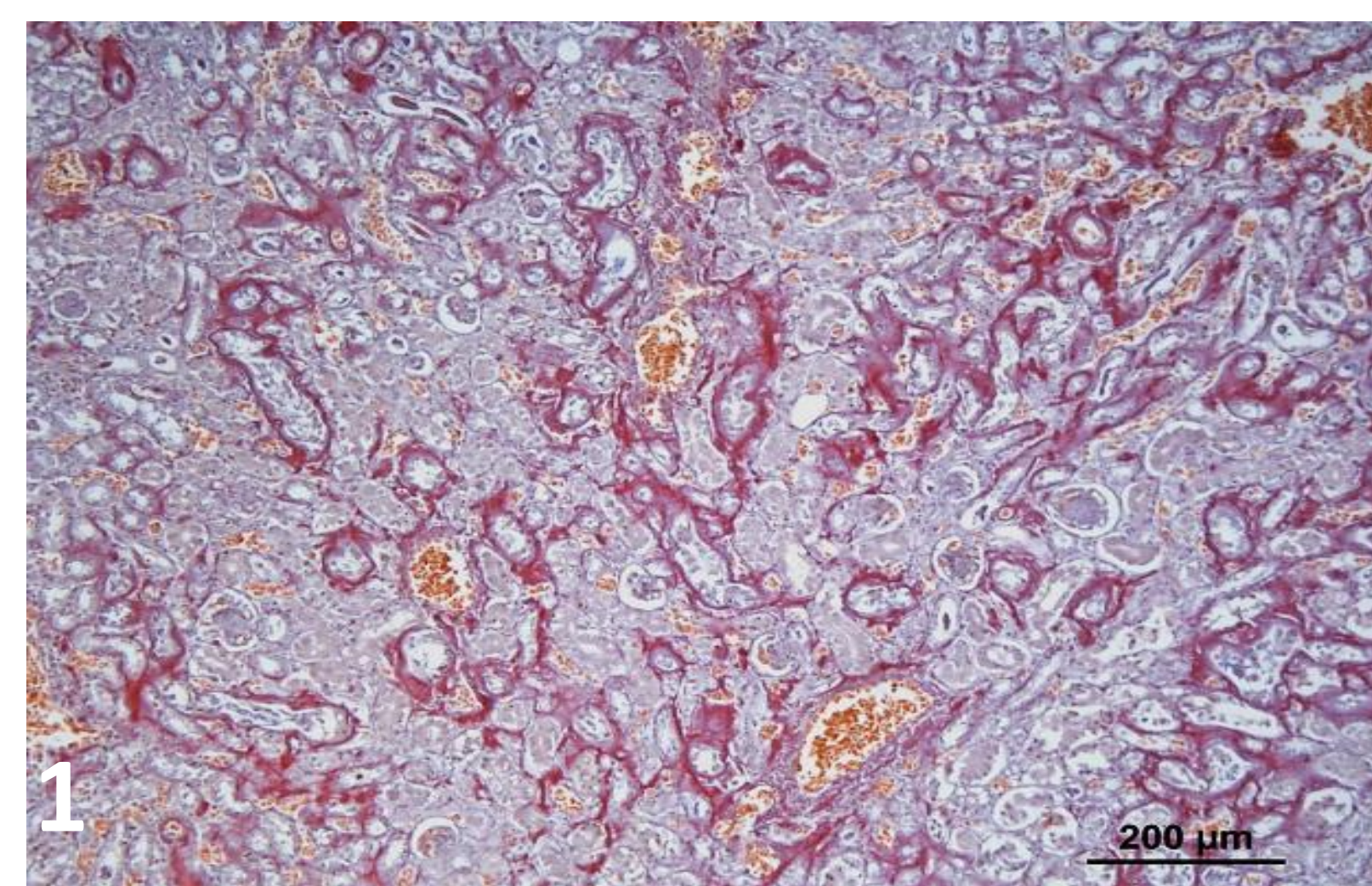
- *Falco peregrinus*, *Gazella dorcas*, *Bos primigenius*, *Capra aegagrus*, *Ovis aries*, *Felis silvestris* and *Canis lupus*

Selection of the organs

- Kidney, spleen and liver

Histopathologic techniques

- Hematoxylin/eosin
- Congo Red
- Immunochemistry
 - Non-commercial rabbit polyclonal antibody
 - Mouse monoclonal antibody anti-Amyloid A Component
 - Rabbit polyclonal antibody anti-Amyloid Precursor Protein (APP)



1. Kidney; *Falco peregrinus*. Congo red
2. Kidney; *Falco peregrinus*. Viewed in cross-polarized light. Congo red
3. Kidney; *Ovis aries*. Hematoxylin/eosin
4. Kidney; *Ovis aries*. Immunochemistry stain using a non-commercial rabbit polyclonal antibody (1:2000)

Conclusions

- Pathogenesis and clinical signs of amyloidosis vary in the different animal species, depending on the cause. Congo Red is still the most used stain to diagnose amyloidosis.
- A transmission between species has been proved. However, the infection by ingestion of contaminated meat is unlikely.
- The best antibody to detect amyloid material is a non-commercial polyclonal rabbit antibody developed by the University of Zaragoza. The best results are obtained with a 1:2000 titer.
- The immunochemistry technique was only effective in *Bos primigenius*, *Capra aegagrus* and *Ovis aries*.

References

1. Blancas L and Ramirez M. *Systemic Amyloidoses* Annual Review of Biochemistry 2013; 82: 745–774
2. Caughey B and Baron G. *Are cheetahs on the run from prion-like amyloidosis?* Proceedings of the National Academy of Sciences of the United States of America 2008; 105: 7113–7114
3. Gruys E. *Protein folding pathology in domestic animals* Journal of Zhejiang University 2004; 5(10): 1226–1238
4. Ménsua C, Carrasco L, Bautista M. J, et. al. *Pathology of AA Amyloidosis in Domestic Sheep and Goats* Veterinary Pathology 2003; 40:71–80
5. Murakami T, Inoshima Y, Ishiguro N. *Systemic AA amyloidosis as a prion-like disorder* Virus Research 2015; 207: 76–81.
6. Murakami T, Ishiguro N, and Higuchi K. *Transmission of Systemic AA Amyloidosis in Animals* Veterinary Pathology 2014; 51(2): 363–371
7. Westermark G. T, Westermark P. *Serum amyloid A and protein AA: Molecular mechanisms of a transmissible amyloidosis* FEBS Letters 2009; 583: 2685–2690
8. Woldemeskel M. *A Concise Review of Amyloidosis in Animals* Veterinary Medicine International 2012; 2012: 1–11